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THE SPONTANEOUS OXIDATION OF ARSENICAL DIPPING FLUIDS.

By AUBREY V. FULLER,
Assistant Chemist, Biochemic Division.

INTRODUCTION.

During the past year and more there has devolved upon the Biochemic Division of this bureau the work of executing numerous analyses of samples of arsenical dipping fluids used by the Zoological Division for experimental purposes in connection with the eradication of cattle ticks.¹

The baths in question were prepared in a manner similar to that described in Bureau of Animal Industry Order 178, page 9, issued March 17, 1911, the essentials of the method being to mix pure arsenic trioxid with an excess of sal soda, and to boil this mixture with water for 15 minutes "or longer if necessary to effect complete solution of the arsenic." The solution is then cooled, pine tar added, and the volume brought to 500 gallons by the addition of clear water. The bath thus prepared should contain in solution sodium arsenite equivalent to approximately 0.24 per cent of arsenic trioxid.

The observation having been made that in several instances batches of used dip which had been allowed to stand in the vats for varying periods of time showed an apparent loss of arsenic, a special study was undertaken to determine if possible the conditions under which this loss took place.

METHODS OF ANALYSIS.

METHOD A.—ARSENIOUS OXID PRESENT AS SUCH.

Fifty cubic centimeters of the dip was pipetted into a separatory funnel of 300 c. c. capacity, acidified with dilute sulphuric acid, and the tar removed by extraction with ether. Comey² states that ether

¹ The results of these experiments are reported in a paper by Ransom and Graybill in the Twenty-seventh Annual Report of the Bureau of Animal Industry (now in press). The details of the experiments are given more fully in another paper by the same authors which is to be issued as Bulletin 144 of the Bureau of Animal Industry.

² A Dictionary of Chemical Solubilities, 1896, p. 30.

extracts almost no arsenic trioxid from a saturated solution containing free sulphuric acid. The aqueous layer was run into a second funnel and extracted with a fresh portion of ether, the ether remaining from the first extraction being washed three times with 25 c. c. portions of 2 per cent sulphuric acid. The ether extraction was repeated until the upper layer in the last funnel appeared colorless on separating, showing complete removal of the tarry matter. Three extractions have been found sufficient in most cases.

The acid washings were put through the ether residues in proper sequence, and finally the aqueous solutions were combined in an Erlenmeyer flask and the dissolved ether was expelled by heating on the steam bath. This latter step may not be necessary, but seems indicated in view of the fact that water containing sodium bicarbonate and saturated with ether yields a precipitate with iodin solution. The liquid was then cooled, made alkaline with sodium bicarbonate, and titrated with tenth-normal iodin, using starch paste as the indicator. One cubic centimeter of tenth-normal iodin equals 0.00495 gram arsenic trioxid.

METHOD B.—TOTAL ARSENIC.

Fifty cubic centimeters of the dip was introduced by means of a pipette into a 100 c. c. measuring flask, acidified with dilute hydrochloric acid, using a drop of methyl orange to read the change, then made to volume with water and thoroughly mixed. The solution was then passed through a quantitative filter and after rejecting the first portions of the filtrate 20 c. c. aliquots of the filtrate were taken for analysis.

From this point the method of Andrews and Farr¹ was employed. This method is based upon the well-known fact that arsenic is reduced to the metallic state by stannous chlorid in strong hydrochloric acid solution. These authors use a reagent consisting of concentrated hydrochloric acid containing 20 grams of stannous chlorid crystals and 40 grams of tartaric acid to the liter, the latter being added to prevent the co-precipitation of tin.

This reagent is mixed with the solution under examination in large excess, and kept at a temperature of 35° to 40° C. for three hours, in a flask provided with a tightly fitting glass stopper. The liquid is then filtered off through a Gooch crucible, the precipitated arsenic after washing with hydrochloric acid and water is returned together with the asbestos mat to the original flask, and the metal is dissolved in an excess of standard iodin solution in the presence of sodium bicarbonate or phosphate. Finally the excess of iodin is determined by titration with either arsenious acid or sodium thio-

¹ American Journal of Science, vol. 27, No. 160, p. 316, 1909.

sulphate. From the amount of iodin consumed the weight of arsenic trioxid is calculated, the reaction taking place in accordance with the following equation:



The method was found to yield good results when the directions of the authors were closely followed, particular attention being paid to the concentration of hydrogen chlorid in the reacting mixture.

APPARENT LOSS OF ARSENIC IN SAMPLES FROM THE FIELD.

The accompanying table embodies the data obtained in connection with the examination of samples of dips exhibiting the peculiar change.

Results of examination of samples of dip.

SERIAL NO. 777.

Date.	Per cent actual As_2O_3 .	Remarks.
Not recorded	0.040	Stood in vat. No analysis of original sample, but made to contain 0.24 per cent As_2O_3 .

SERIAL NO. 778.

October, 1909.....	0.225	Original sample.
December, 1909.....	.020	Stood in bottle in laboratory.

SERIAL NO. 887.

June, 1910.....	0.231	Stood in vat about 1 month before sample was taken.
October, 1910.....	.011	Stood in bottle in laboratory.

SERIAL NO. 971.

June 23, 1910.....	0.217	Original sample.
July 14, 1910.....	.218	Stood in vat.
September 15, 1910.....	.040	Do.
October 20, 1910.....	.020	Do.

In the examination of these samples method A was employed, which it will be seen is designed primarily for the determination of arsenic present in the trivalent state and hence when applied to the determination of total arsenic must rest upon the assumption that the metal is present entirely in this condition. That this assumption was warranted seemed indicated in view of the fact that it is generally considered that arsenic trioxid is not prone to rapid oxidation when subjected to moderate boiling with alkali carbonates, though this tendency is quite marked when the hydroxids are used.

However, it was deemed advisable to verify this hypothesis experimentally. With this object in view, determinations of arsenious

arsenic and total arsenic were made on several samples of freshly prepared dips and concordant results were obtained, thus establishing the validity of the assumption.

As an additional check, the total arsenic was determined in serial No. 971, which is selected as a typical illustration of the progressive loss of arsenious arsenic, with the following results:

Original concentration of arsenious arsenic, June 23, 1910, 0.217 per cent As_2O_3 .

Concentration of arsenious arsenic, July 14, 1910, 0.218 per cent As_2O_3 .

Concentration of arsenious arsenic, September 15, 1910, 0.040 per cent As_2O_3 .

Concentration of arsenious arsenic, October 20, 1910, 0.020 per cent As_2O_3 .

Concentration of total arsenic, October 20, 1910, 0.217 per cent As_2O_3 .

Since the figure for total arsenic in solution was in exact agreement with the original value, it is evident that none had been actually removed from the bath.

EXPERIMENTAL WORK.

Several hypotheses suggested themselves to the writer as possibly serving to explain the alteration observed in the samples in question. These hypotheses, together with the experimental work done in testing their adequacy, will now be discussed.

ORGANIC COMBINATION OF THE ARSENIC.

One explanation which was considered was that the arsenic had in some obscure manner formed an organic compound with certain constituents of the pine tar present, and that this compound was soluble in ether and was thus lost when the first method of analysis was employed. Though this possibility was considered quite remote, it was decided to subject it to experimental examination. To this end a 20 c. c. portion of a sample which had exhibited the change was shaken out with ether in the manner described and a determination made of the total arsenic in the aqueous layer. The result was in agreement with that obtained by the total arsenic method previously described, thus removing the possibility of explaining the phenomenon on these grounds.

OXIDATION BY AIR IN THE PRESENCE OF PINE TAR.

It having been shown by the previous experiment that the arsenic was not lost through organic combination, the only remaining alternative was that it had undergone oxidation. That such was the case was shown in the following manner:

A sample of serial No. 971¹ was extracted with ether as previously described and the arsenic precipitated with magnesia mixture. The voluminous precipitate of magnesium ammonium arsenate with

¹ Compare results on pp. 3 and 4. This analysis was made subsequent to October 20, 1910.

presumably some phosphate was filtered off, washed, dissolved in hydrochloric acid, and after the addition of potassium iodid¹ the arsenic was precipitated as the trisulphid by means of hydrogen sulphid.

The sulphid was collected on a filter, washed with water containing hydrochloric acid and hydrogen sulphid, and then dissolved by heating with sodium hydroxid solution with the addition of 50 c. c. of hydrogen peroxid. By this treatment the sodium sulpharsenite is oxidized to arsenate and sulphate and the solution may be acidified without reprecipitation of arsenic sulphid.

The solution was next made slightly acid with sulphuric acid, filtered, the filtrate made slightly alkaline with ammonia, and the arsenic again precipitated as magnesium ammonium arsenate. This precipitate was finally ignited and weighed as magnesium pyroarsenate in the usual manner.

The result closely approximated that obtained for total arsenic by the Andrews and Farr method, and served to prove beyond a doubt that the process operative in this instance was one of oxidation.

Although, as previously mentioned, sodium arsenite in the presence of carbonate does not suffer marked oxidation, it was considered entirely possible that such might be accomplished through the agency of the pine tar contained in these samples. Commercial pine tar contains terpenes and other bodies found in oil of turpentine, among which are certain compounds which form oxidation products on exposure to the air. These products readily yield their oxygen to reducing substances, such as sodium sulphite, and to certain organic substances, such as guaiac, in the presence of various enzymes, this property constituting the basis of several chemical tests for establishing the presence of certain of such latter bodies. Hence it was regarded as quite possible that the peroxids or similar bodies formed by the action of air on the pine tar might in turn effect the conversion of the sodium arsenite to arsenate.

To test this suspicion a sufficient quantity of the "dip" was prepared according to directions previously referred to, and the arsenic determined according to method A. The solution was then placed in a tall cylinder and a moderate current of air passed through it for a period of eight days. At the end of this time a second sample was taken and analyzed for arsenite with the result that the value obtained was identical with the first, no oxidation having taken place. The experiment was repeated, using a different sample of pine tar, with the same result.

Evidently, then, the cause of the phenomenon lay elsewhere.

¹ The potassium iodid feature was proposed by De Koninck (*Bulletin de la Société Chimique de Belgique*, vol. 23, p. 88, 1909) for the purpose of hastening the reduction and consequently the precipitation of pentavalent arsenic.

BACTERIAL ACTION.

All attempts to explain the oxidation of the arsenite on a purely chemical basis having proved unfruitful, the action of micro-organisms was next considered.

While numerous species of fungi have been found to possess the property of reducing¹ arsenic compounds to arsin, the author has been unable to find in the literature any mention of the reverse property of effecting oxidation of arsenites.

Although compounds of arsenic have long been used as insecticides and exert marked toxic effects on the higher organisms, the arsenites and arsenates possess but little antiseptic or disinfecting power against the lower fungi, except when present in rather high concentration. Thus, according to Rideal,² Miquel found that there was required 6 grams of arsenic trioxid to the liter to prevent bacterial growth in beef tea, and that 125 grams of potassium arsenate or 9 grams of sodium arsenate were required to accomplish the same purpose. It was therefore not surprising that a sample of an arsenical dip which exhibited the characteristic oxidation contained numerous living bacteria, as was demonstrated by making plate cultures on nutrient agar inoculated with the dip.

Acting upon this clue, the following experiment was carried out to determine if the bacteria present in a sample of used dip could bring about oxidation in a fresh sample and also to determine the conditions under which this oxidation might take place.

A batch of arsenic pine-tar dip was prepared after the manner previously described, 800 c. c. portions were placed in each of 10 flasks having a capacity of 1,500 c. c., and were further treated as shown in detail in the ensuing table. The flasks having been prepared, samples were taken for analysis by withdrawing the cotton stoppers with which they were provided, thoroughly flaming the necks, and pouring about 60 c. c. from each into a 100 c. c. graduated cylinder, from which containers the desired portions were removed by means of a transfer pipette. The flasks were then placed in a dark closet and allowed to remain at room temperature with occasional agitation for one month. At the expiration of this time samples were taken as before and analyzed for arsenious arsenic. In the accompanying table are given the descriptions of the contents of the various flasks, together with the initial and final percentages of actual arsenic trioxid as determined by method A.

¹ Compare W. Kruse, *Allegemeine Mikrobiologie*, Leipzig, 1910.

² Disinfection and Preservation of Food, New York, 1903, pp. 176-177.

Results of examination of samples of dip to determine oxidation of arsenic.

Flask No.	Description.	Initial per cent As ₂ O ₃ .	Final per cent As ₂ O ₃ .
1	Straight dip sterilized ¹	0.236	0.222
2	Straight dip sterilized+20 c. c. used bath No. 971	.225	.197
3	Straight dip+66 c. c. broth sterilized	.221	.207
4	Straight dip+66 c. c. broth+20 c. c. used bath No. 971	.217	.006
5	Straight dip sterilized+50 c. c. raw cattle urine	.215	.190
6	Straight dip sterilized+50 c. c. sterilized ² cattle urine	.223	.198
7	Straight dip+50 c. c. sterilized cattle urine+20 c. c. used bath No. 971	.223	.179
8	Straight dip+60 c. c. broth sterilized+50 c. c. sterilized cattle urine	.214	.188
9	Straight dip+66 c. c. broth sterilized+50 c. c. sterilized cattle urine+20 c. c. used bath No. 971	.209	.011
10	Straight dip sterilized+50 c. c. raw cattle urine+20 c. c. used bath No. 971	.242	.011

¹ Sterilization was accomplished by heating in the autoclave at 12 pounds pressure for 50 minutes.

² Sterilized in the same manner as the arsenic solutions.

Comparison of the above initial and final values for trivalent arsenic develops the fact that although none of the flasks showed absolute freedom from oxidation, the change was most striking in Nos. 4, 9, and 10. These flasks contained in addition to the arsenite-tar solution a suitable culture medium, and each was inoculated with a portion of a dip which had been used in the field and in which oxidation had taken place. In other words, the inoculum contained an organism which when transplanted to a solution containing sodium arsenite and pine tar of the concentration indicated, and in addition a suitable culture medium, was capable of producing almost complete oxidation of the arsenite. That only a slight oxidation was obtained in flask No. 7 may be explained by the fact that the urine through sterilization had been rendered unsuitable as a culture medium, it being the usual practice when this fluid is used for the cultivation of bacteria to accomplish sterilization by passing it through a Chamberland filter.

Since the pine tar was an undesirable constituent of the solutions used for experimental purposes in that it rendered them opaque, thus making impossible observation of clouding due to bacterial growth, it was determined to eliminate it if proof could be obtained that its presence was not essential for the occurrence of the phenomenon of oxidation. To this end a solution of sodium arsenite (0.220 per cent As₂O₃) containing 20 per cent of neutral bouillon¹ was prepared and placed in a liter flask provided with a two-holed rubber stopper and glass tubes. The exterior ends of the tubes were plugged with cotton, and the flask thus fitted was sterilized in the autoclave. After cooling, the flask was inoculated with 20 c. c. of sample No. 971, and connected in series with a wash bottle containing sterile water and one containing concentrated sulphuric acid to which was added potassium bichromate. A slow current of air was

¹ The As₂O₃ and Na₂CO₃ were dissolved by boiling in 50 c. c. water, this solution was added to the bouillon, and the mixture was boiled and filtered in order to remove phosphates, etc., which would otherwise precipitate during the process of sterilization. The filtrate was then made to the required volume with distilled water and the arsenic content established by titration with iodin solution as described.

passed first through the bottle containing the acid to render it sterile, then through the water to saturate it with moisture, and finally through the culture flask.

At the end of 10 days the arsenic had been practically completely oxidized, thus establishing the fact that the pine tar played no essential part in the reaction.

Further experiments have shown that it is possible to bring about oxidation by inoculating a flask with so small a quantity as five loopfuls of a solution which has shown the oxidation, and to carry this inoculation with its attendant oxidation progressively from one flask to another.

Attempts have been made to isolate, by the ordinary bacteriological methods, the organism or organisms causing the oxidation, but so far they have proved unfruitful. This work will be continued as the opportunity presents itself.

SUMMARY AND CONCLUSIONS.

1. It has been shown in the present paper that sodium arsenite may undergo oxidation to arsenate through the agency of a micro-organism or organisms not yet identified.

2. This oxidation may be quite rapid and in time proceeds practically to completion. The velocity of the reaction is dependent, of course, upon the number of the organisms introduced and upon their rate of multiplication, the latter factor being determined by the nature and quantity of organic matter present, the temperature, and, in short, all the agencies which influence bacterial growth.

3. Inasmuch as the organism or organisms in question have not as yet been identified, it is impossible to state at present just how they gain access to the baths. There are several channels through which the inoculation might be effected, namely, through the water used in preparing the solutions, through the air, or through excrementitious or other matter derived from the cattle which pass through the dip.

4. In view of the fact that the conditions to which the baths are exposed in actual practice are necessarily subject to wide variations, it is difficult to set a limit to the time which a given dip might be allowed to remain idle without undergoing alteration. However, it would appear safer to discard any batch of dip which is more than a few weeks old unless there is positive evidence that it retains its original concentration of actual sodium arsenite.

5. Efforts will be made to isolate the causative agent of this phenomenon and to study its properties.

Approved.

JAMES WILSON,

Secretary of Agriculture.

WASHINGTON, D. C., September 9, 1911.

